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**FAX N° 00 49 89 2399 4465****CONFIRMATION BY COURIER**

Re: International Patent Application No. PCT/ES03/00063  
VITATENE, S.A., et al.

**Further reply to written opinion pursuant Rule 66.4 PCT**

Further to our previous reply to the WO issued by the EPO on 19.03.04, we have maintained a phone conversation with the examiner in charge of present IA to discuss some outstanding objections.

As consequence of that phone talks, we agree to further amend the set of claims under examination, pursuant Art. 34 PCT to accomplish with examiner suggestions. Precisely, we have further amended claim 1 by adapting previous drafting to the wording used in the specification as formerly filed. Thus, "...and then carrying on the selection of astaxanthin overproducing mutant strains among the mutants selected after the first selection step, by a further selection step based on a technical feature, selected among: ....", has been replaced by "...and then astaxanthin overproducing mutant strains were selected subsequently as a function of their yield on solid medium measured as: ....". The amended wording has been taken from pg. 9 lines 37-38 of the specification as formerly filed.

Moreover, "...i. deeper red coloration..." has been replaced by "...i. deeper red coloration than the parent strain..." as stated in pg. 10, lines 2-3 of the specification. Also "...iii. production of astaxanthin at elevated temperatures...." it has been replaced by "...iii. production of astaxanthin at temperatures over 20°C...." which it is the range top value disclosed also in pg. 10, lines 15-17 and stated as usual temperatures for producing astaxanthin by fermentation in the prior art.

Concerning the suggestion made by the examiner of limiting claim 1 by referring the growing of mutant strains, instead on solid medium to YEPDA medium, we have respectfully considered that pg. 9 lines 16-18, wherein YEPDA medium is clearly defined as solid medium, it would bring enough support for the generalization made in claim 1, without further restrictions being necessary.

We have also changed the multiple dependency of claims 11, 13 and 15 as pointed out kindly by the examiner, being now each one of the aforesaid claims dependent only on claim 8.

In relation to the lack of inventive step of claims 24-27 we would like to highlight the following facts. The technical problem solved by the invention it is to find an alternative food biomass for feeding animals which confers to the flesh of the animals (fish, salmons, trouts, mainly) red-orange colour pigmentation, which makes them more attractive for consumers. Closest prior art may be either D1 or D2. D1 (see Table 9, diet A at pg. 37) shows a pigmentation of fish flesh after 93 days of feeding the trouts with its biomass mixed up with ethoxyquin, of 2.01 ppm /wet wt. The invention shows, using the biomass containing mutants, also mixed up previously to be added to the tank wherein the trouts are being grown with the antioxidant ethoxyquin (see example 13 of the specification), a pigmentation reaching an average of 3.5 ppm ( $\mu\text{g/g} = \text{ppm}$ ), that is 75% higher than the pigmentation achieved in D1 in analogous conditions. Moreover that pigmentation of the trouts' flesh was obtained by the invention in only 2 months instead the 93 days needed in D1. Therefore, the biomass of the invention seems to have surprisingly a stronger pigmentation power and a faster assimilation into fish flesh. By departing from the teaching of D1, the man skilled in the art would try to improve the pigmentation either by using further additives as soy lecithin or safflower oil (diets B or C, respectively of table 9). Also, by combining D1+D2 the man skilled in the art would be persuaded to hydrolyze the cell walls of the yeast mutants by enzymatic lysis with *Trichoderma harzianum* to improve the availability of astaxanthin pigment. Accordingly, the solution found in present invention, biomass containing mutants with more intense colouring features, without the use of much additives and which promotes faster assimilation of the pigment into fish flesh thus making unnecessary

to disrupt said biomass to get that improved pigmentation, it is not presently suggested in the prior art, either departing from D1 or D2 or by a combination of those 2 documents. Inventive step of amended claims 24-27 should hence be acknowledged.

Yours faithfully,

E L Z A B U R U

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Manuel Illescas

Annex:

- Newly amended set of claims 1-27